The Rejection under 35 U.S.C. § 103

The rejection of claims 12-18, 20-21, 38-41 and 42-43 under 35 U.S.C. § 103, as being obvious over Kendall (U.S. Patent No. 5,026,728) in view of Caughey (1983 article) or Gibson (1980 article) or McFarlane (U.S. Patent No. 4,455,298) is respectfully traversed.

Applicants maintain their position that the original data of record in the specification establish significant unexpected advantageous properties of the combination composition of matter of applicants' invention, which would not have been expected from the prior art. Because the scope of the showing was questioned (and alleged inadequate) in the Office Action, these data are supplemented with further proof of unexpected advantages in the attached Declaration under 37 CFR 1.132 by Dr. John Lawson, discussed in more detail below. The data considered as a whole establish unexpected, advantageous properties representative of the full scope of the claimed subject matter and, thus, prove the nonobviousness of the claimed invention.

The data as a whole establish that the combination of DMG and Perna provides a significant and unexpected advantageous result over what would have been expected from the prior art teachings pertaining to the use of either DMG or Perna alone. Applicants' disclosure and data show that the combination exhibits properties useful for treating human systematic lupus erythematosis (SLE), hereinafter "lupus" and/or providing a particular combination of immune response effects, neither of which could have been expected by combining the prior art taught effects of DMG and Perna administered alone. The data, thus, clearly and convincingly proves nonobviousness even if there is a *prima facie* case for making the combination of DMG and Perna in the manner recited in the claims. While Kendall '728 provides a generic teaching which includes lupus in a list of autoimmune diseases treatable

using DMG, the secondary references directed to the use of Perna provide no suggestion to use Perna for treating lupus or that Perna would effect the kind of immune response shown by applicants. Thus, there would be no expectation in the art that a combination of DMG and Perna would be useful for treating lupus and/or effecting the shown immune response. Accordingly, the data reasonably establishing the activity of the combination of DMG and Perna for treating lupus and/or for effecting the shown immune response are, in fact, unexpected results.

In the Office Action, it was alleged that the advantages shown are only "modest" and cannot be considered unexpected. Applicants respectfully disagree. The data show significant activity for treating lupus conditions in the prevalent animal model used for modeling treatment of lupus in humans, as established in Dr. Lawson's newly submitted declaration (see pages 1-2 and the beginning of the Discussion section). The cited prior art suggests no such activity for the combination of DMG and Perna; thus, the results are highly significant.

It was further alleged that the claims are not commensurate in scope with the unexpected results shown and that the particular experimental model, product composition and method of administration by which the unexpected results were obtained must be considered. The implication from the Office Action appears to be that the showing of unexpected results would only be convincing if the claims were limited to the exact specific animal model, specific composition and specific dosage shown by the comparative experiments. The law, however, does not dictate such an outcome. The law requires only that the unexpected results be significant and reasonably representative of the scope of the claimed subject matter; see, e.g., In re Kollman, 201 USPQ 193 (CCPA 1979). As to use of

the specific animal model, such is warranted by the fact that this is the model most prevalently used in the art for modeling the human lupus condition. As for the specific composition, the claims are also so limited, i.e., to a combination of DMG and Perna and to an enteral but not parenteral mode of administration. As to the dosages, the data from the specification is supplemented with that in Dr. Lawson's declaration wherein DMG and Perna are administered in varied concentrations and a new larger test and control group of the MRL/lpr mice are used.

The showings here are reasonably representative of the entire claimed scope at least because: 1) MRL/lpr mice are the accepted model in the art for human lupus conditions, 2) the prior art gives no hint of anti-lupus activity for Perna, thus any such activity demonstrated by the combination of DMG and Perna could not have been expected, and 3) the combined data of the specification and Dr. Lawson's declaration shows such advantages in a significant number of tests.

The new declaration of Dr. Lawson shows that, unexpectedly from the cited prior art, the combination of DMG and Perna is effective for treatment of nephritis in MRL lpr/lpr mice and, thus, reasonably expected to be effective against human lupus. When they are administered together, DMG and Perna significantly amplified the down regulation of IgG-2a as compared to the DMG or Perna alone (see, e.g., Figures 7 and 10). Further, the combination of DMG and Perna significantly suppressed the development of glomerulonephritis and lymphadenopathy in MRL lpr mice (see, e.g., Tables 8-11). These results suggested that the combination of DMG and Perna ameliorates the lupus-like autoimmune disorders by modulating Thl, which in turn results in skewing positively of the immune response in MRL/lpr mice. The DMG and Perna combination clearly suppressed the

production of IgG-2a level in serum (see, e.g., Figure 6). It did not change that of IgGl (see, e.g., Figure 11) but increased the production of IgG3 in serum (see, e.g., Figures 11 and 12), thus, the total concentration of immunoglobulin essentially remained the same. DMG and Perna showed significant effects on lymphadenopathy. Average lymph nodes weight for mice treated with the highest concentration of DMG (200 mM) plus Perna was reduced to half compared to control mice. This reduction was also seen in other treatment groups with 100 mM DMG but not as significant as in 200 mM treated mice. Animals treated with different concentrations of Perna plus 150 mM DMG showed similar results (see Tables 10 and 11). Thus, Dr. Lawson concludes, it is shown that the immunomodulation resulting from the combination of DMG and Perna significantly reduces the progression of glomerulonephritis and pathological IgG-2a antibody levels and significantly reduced the pathological enlargement of the lymph nodes, these properties being shown in the prevalent mouse model being of high significance to treating SLE patients with glomerulonephritis. Such significant property of the DMG and Perna combination could not have been expected by one viewing the previous reports on uses of DMG and Perna.

The previously discussed data shown in the instant specification can be summarized as follows. It provides side-by-side data comparing immune responses in the established prevalent animal model for human lupus. The data show that the combination of DMG/Perna provides an advantageous result, which is not merely the expected combined effect of DMG and Perna alone but an effect, which is **different in kind** than what would have been expected in the prior art. Particularly, the data showed:

- that il-10 cytokine production is **increased** with DMG alone and only slightly decreased, if any, with Perna alone,

- that the il-10 production is surprisingly **significantly decreased** by the combination of DMG and Perna according to the invention,
- that TNF-alpha levels are **decreased** with either of DMG or Perna alone but, surprisingly, **increased** by a combination of DMG and Perna,
- that the DMG/Perna combination, over either of DMG or Perna alone, in reduces CD8 and CD19 lymphocytic markers,
- that the DMG/Perna combination, over either of DMG or Perna alone, reduces anti-dsDNA antibody levels and anti-ssDNA antibody levels.

The significance of these results (as discussed in the specification, Example 2; see page 11, first two full paragraphs) includes that they are indicative of a shift from a Th2 type response to a Th1 response for these cytokines. Such a shift is of high significance to the immune response generated, as discussed in the previous response.

That the references may suggest that both Perna and DMG are anti-inflammatories does not end the inquiry, as implied in the Final Office Action. Even if a prima facie case of obviousness is established, evidence of nonobviousness must be considered when making the ultimate determination of obviousness. It is certainly incorrect, as a matter of law, to ignore evidence of nonobviousness because it is believed a prima facie case of obviousness is established. See, e.g., In re Papesch, 137 USPQ 143 (CCPA 1963), and In re Oetiker, 24 USPQ2d 1443 (Fed. Cir. 1992). One of ordinary skill in the art may have expected that, since DMG and Perna were both known as anti-inflammatories, their combination would have been expected to provide the combined effect of their anti-inflammatory properties. However, if applicants can show that their combination provides other properties that would not have been expected by one of ordinary skill in the art, they are deserving of patent

protection. Applicants have done so in the above-discussed data. By such showing, applicants have provided an advance in the art deserving of patent protection since there is no actual teaching of the combination, as claimed by applicants, and applicants have shown their combination to provide advantages that could not have been expected by one of ordinary skill in the art. It is only through applicants' invention that these significant advantages of the combination of DMG and Perna are known. That the prior art might have suggested the two components could be combined to possibly provide an anti-inflammatory property does not lessen applicants' contribution of actually combining them, as claimed, and discovering the new and unexpected properties thereof, the only discovery which provides an actual basis for one to make the combination.

The Office Action continues to dismiss the evidence from the Belkowski disclosure, which actually teaches away from the claimed invention. It is alleged in the Office Action that the Belkowski teachings are not applicable because they relate to an artificial model. Applicants strongly disagree with the dismissal of this further evidence of nonobviousness. The model which Belkowski used is one that, at least he, considered useful in assessing, for human application, the properties of Perna and DMG individually and in combination. Belokowski obviously was not conducting his research with the objective of treating arthritic rats but for treating arthritis in humans. Thus, Belkowski's teachings are highly significant as to what one of ordinary skill in the art would have expected from the combination of DMG and Perna. In fact, they are the only teachings in the prior art, which relate to any actual combination of DMG and Perna - albeit in a combination distinct from that of the instant claims. Contrary to backing up what may have been the prima facie view in the art - i.e., that combining the anti-inflammatories would provide a heightened effect over either alone -

Belkowski provides at least some suggestion that the combination of Perna and DMG is less effective than Perna alone. This evidence is clearly relevant to what one of ordinary skill in the art was taught about combining DMG and Perna. It must be considered and cannot be ignored. This teaching away adds to applicants' showing of nonobviousness.

For all of these reasons at least, the rejection under 35 U.S.C. § 103 based on the combination of the Kendall '728 reference with Caughey or Gibson or McFarlane '298 should be withdrawn.

The Rejection under 35 U.S.C. § 112

The rejection of claims 12-18, 20-21, 38-41 and 42-43 under 35 U.S.C. § 112, first paragraph, for lack of written description, is respectfully traversed.

The recitations of "enteral but not parenteral administration" forms and "not sterilized" forms in the claims are not new matter. Applicants clearly stated in their last response that the recitations were supported by the disclosure, albeit not literally. If they are supported by the disclosure, it follows that they are not new matter. Further, contrary to the inference in the Office Action, the claim language need not be literally recited in the specification in order to be supported. The law is well established that the subject matter of a claim need not be described in the specification literally or "in ipsis verbis" in order for the specification to satisfy the description requirement of 35 U.S.C. § 112, first paragraph. See, e.g., In re Lukach, 169 USPQ 795 (CCPA 1971); Kennecott Corp. v. Kyocera International, Inc., 5 USPQ2d 1194, 1197 (Fed. Cir. 1987); Martin v. Johnson, 172 USPQ 391 (CCPA 1972); and In re Wertheim, 191 USPQ 90, at 98 (CCPA 1976).

The original disclosure (e.g., page 5) conveys to one of ordinary skill in the art that, for both components, the "enteral" and "parenteral" forms of administration are both

optional. When a disclosure recites an element as being optional, it adequately describes under 35 U.S.C. § 112, first paragraph, both the option of inventions meeting the recitation of that element and the option of inventions not meeting the recitation of that element; see, e.g., Ex parte Cordova, 10 USPQ 2d 1949 (Bd. App. 1988), and Ex parte Wu, 10 USPQ 2d 2031 (Bd. App. 1988). Since enteral and parenteral forms are both described as optional, the specification described administration by enteral, not enteral, parenteral and/or not parenteral forms. Accordingly, the option of "enteral but not parenteral" is adequately described.

The option of the compositions being "sterilized" is specifically stated in the disclosure at page 5, lines 28-29, thus the "not sterilized" option is clearly disclosed. Further, even if not literally recited, it is inherent in the specification since one of ordinary skill in the art knows well that parenteral forms must be sterilized while enteral forms need not be.

As further proof of the correctness of the above position and proof of what one of ordinary skill in the art would understand as being described from applicants' disclosure, applicants submitted a Declaration under 37 C.F.R. § 1.132 by co-inventor Dr. Roger Kendall who is knowledgeable as to the meaning in the art of the terms pertaining to routes of administration of the components at issue. The facts established in this declaration were confirmed in Dr. Lawson's declaration submitted herewith. Thus:

- one of ordinary skill in the art would interpret the term "enteral" as being distinguished from "parenteral,"
- it is known that enteral and parenteral administration forms have very different requirements, e.g., parenteral forms must be sterile,
- enteral forms need not be sterile,

- that the specification thus necessarily describes forms for enteral administration which are not suitable for parenteral administration (e.g., they are not sterilized) as one option, and
- the specification actually provides Examples where the administration form is suitable for enteral administration but not for parenteral administration.

In the Final Office Action, Dr. Kendall's declaration was discounted on the bases that he was not an established "expert" and that he was an inventor with an interest in the outcome of the case. Applicants strongly protest. Dr. Kendall's declaration clearly set forth that his statements were made based on his knowledge of the art and that therefore he could attest to how one of ordinary skill in the art would have considered the disclosure. How one of ordinary skill in the art would have considered the disclosure is the issue at hand and the issue addressed by the declaration, thus, he is established as an "expert" for this purpose. Dr. Lawson further confirms these facts. That Drs. Kendall and Lawson are the inventors of this application does not make their declarations irrelevant. It is quite common for inventors to submit declarations supporting the patentability of their inventions to the PTO and such declarations are generally given full consideration. There is no evidence of record or implication that the inventor/declarants are being less than forthright and it must be remembered that the declarations include the concluding paragraphs whereby the strict consequences for willful false statements is set forth. Absent some objective reason given by the PTO for why the inventor/declarants' statements are not believed true, it is submitted that they must be fully considered.

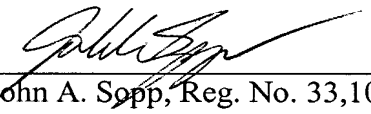
Accordingly, applicants re-assert that the evidence of record verifies that one of ordinary skill in the art would consider the invention of the instant claims to be described in the original disclosure.

For the above reasons, it is urged that the 35 U.S.C. § 112, first paragraph, rejection should be withdrawn and no similar rejection should be made against the current claims.

It is submitted that the application is in condition for allowance. But the Examiner is kindly invited to contact the undersigned to discuss any unresolved matters.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,



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Attorney Docket No.: FSC-6

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Roger KENDALL et al.

Group Art Unit: 1644

Application No.: 09/316,001

Examiner: Ewoldt, G.

Filed: May 21, 1999

For: METHODS AND COMPOSITIONS FOR MODULATING IMMUNE RESPONSE AND FOR THE TREATMENT OF INFLAMMATORY DISEASE

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, John Lawson, being duly warned, declare that:

I am a citizen of the United States, residing in Clemson, South Carolina.

I am an inventor of the above-captioned application and am, therefore, familiar with the invention described therein and with the grounds for rejection made against the claims of the application in the Office Action mailed October 17, 2002, from the U.S. Patent and Trademark Office.

My attached "BIOSKETCH" establishes my expertise in the field of microbiology and pharmacology to which this invention relates.

The following experiments were conducted by me or under my supervision by my research group at Clemson University.

MRL lpr/lpr mice spontaneously develop a lupus-like auto-immune disease resembling human systematic lupus erythematosus (SLE) characterized by immune complex mediated glomerulonephritis, enlargement of the spleen and lymph nodes, and the production

of auto-antibodies such as anti-DNA antibodies. The MRL model is widely used as an animal model for the study of human lupus erythematosis. Alterations in both Th1 cellular and Th2 humoral immune parameters and the clinical and histological progression of the disease mimic very closely those patterns characteristic of the human syndrome. Accordingly, favorable affects of agents on these symptoms in MRL lpr/lpr mice would be reasonably expected to indicate use of such drugs for treating human lupus erythematosus.

In our studies, discussed in more detail below, significantly lower levels of anti-nuclear antibodies were determined in animals receiving orally more than 100mM dimethyglycine (DMG) measured in their drinking water in addition to a Perna-mouse chow mixture which contained about 40% Perna powder compared to control MRL lpr/lpr mice.

Both DMG and Perna alone showed an immunomodulating effect and a lowering of IgG-2a antibody levels. But the DMG plus Perna combination not only significantly lowered IgG-2a serum levels as compared to controls by 8 weeks but also compared to either DMG or Perna alone by the 16th week when lupus-like clinical symptoms become evident in the controls. High concentrations of serum IgG-2a are generally associated with pathological lesions in auto-immune diseases such as lupus erythematosus.

Control MRL lpr/lpr mice are characterized by pronounced lymphadenopathy. Significant reduction of lymphadenopathy was observed in animals fed a combination of DMG and Perna, as determined by weight measurements on sacrifice.

In addition, control mice exhibited extensive deposition of immunoglobulin in the kidney indicative of severe immune complex formation while little if any immune complex deposition was observed in animals fed a combination of DMG and Perna.

CLINICAL

Clinical observation

The treatment and control groups of mice are characterized in Tables 1-4. The effect of DMG (in acidified drinking water) and Perna (41% mixed with standard mouse chow) on the induction of lupus-like disease in MRL lpr/lpr mice was determined by comparing the weight, skin lesion, and other clinical changes. Mice were weighed once a week, and the average weight for MRL mice was about 35 grams at the beginning of the experiment (see Tables 16 and 17).

Skin lesions were observed in control group animals (see Figure 1), especially after the lupus syndrome begins in MRL mice (12-weeks-old). A similar ulcer, however, was also seen in the 200 mM DMG treated animals We have previously observed that very high concentrations of DMG (200 mM and above) leads to symptoms of cachexia or "wasting syndrome" with concomitant ulceration coincident with the stimulation by DMG of both tumor necrosis factor and Il-1. This stimulation of inflammatory cytokines is very beneficial in the treatment of metastatic lesions which tend to suppress the secretion of these cytokines. On the other hand, these inflammatory mediators must be closely monitored in an already inflamed condition such as SLE.

Internal bleeding was another symptom seen in MRL mice (see Figure 3). This could be one of the gastrointestinal manifestations in SLE. In some other cases, the abdominal area is full of liquid, which could be caused by kidney failure.

Effect of DMG and Perna on lymphoid organs

MRL/lpr mice are also characterized by pronounced lymphadenopathy or splenomegaly. MRL/lpr mice administered DMG and Perna from 7 weeks of age were sacrificed at 16 or 22 weeks of age. At the time of sacrifice, mice were analyzed for spleen and lymph node (LN) weights. No splenomegaly was noticed in treated mice. In all three treatment groups reduced lymph node weight was observed, when compared with control mice. Weights of LNs (combined axillary, inguinal, cervical, mesenteric) were marginally increased in the water only control (CH) group (see Tables 5 and 6). The LNs weight showed a two-fold increase in that group of animals at 22 weeks of age. There is not much difference between weight of LNs or spleens in the three different combined DMG and Perna treatment groups. However, in the treatment group with the highest concentration of DMG with Perna (see Tables 5 and 6) slightly lower weights of LNs and spleen were observed.

In a subsequent experiment, 9-week-old male MRL/lpr mice were treated with DMG at a constant concentration (150 mM) and various amounts of Perna. It should be noted that two control animals and two treated ones died (having displayed weight loss) before the termination of experiment and thus were excluded from further analysis. The lymph node weights were slightly reduced in all the three treatment groups at 16 weeks of age. There were no changes in spleen weights at that period. At 22 weeks of age, no significant difference was observed in spleen and LNs weight of T1 and control group (see Tables 10 and 11). However, the treated group with highest amount of Perna (T_3) combined with DMG (150 mM) demonstrated the lowest weights of LNs and spleens (see Table 11).

Kidney Pathology

The kidneys from 16- and 22-week-old mice for each treatment group were collected and sectioned. Kidney sections of all the mice were examined histologically and by immunofluoresence staining (IF). The 5-month-old mice exhibited some mesangial proliferation and sclerosis associated with glomerular deposits of IgG segmentally in mesangium as well as the glomerular capillary wall (Figure 15). In a control mouse, an additional lesion was found in the tubulointerstitial area characterized by variation in tubular size, some focal loss of tubular epithelial cell, numerous casts, peritubular fibrosis, and one area of questionable vasculutis (Figure 15b). Glomerular inflammatory cells were graded "rare" when 0-1 cells were seen per flomerular, and "occasional" when 1-2 cells were seen per glomerulus (Figure 16). Mesangial proliferation was graded 0-3+, in which 0 was negative, 1+ was 25-50% of the mesangium, 2+ was 50-75%, and 3+ was the entire mesangium (Figure 16).

Blood Urea Nitrogen (BUN) was measured by using Azosticks (see Figure 4). No significant difference was observed between treatment and control groups in both BUN and Kidneys WT. Comparison between these three different treatment groups shows slightly lower amount of BUN and kidneys WT for treatment groups with higher concentration of DMG (TC) (see Tables 12 and 13). Similar results on average BUN and Kidney WT were observed for treatment groups with average concentration of Perna (T2) (see Tables 14 and 15).

Serologic characteristics

The levels of antibody at different times were also investigated throughout the study. At sacrifice (4-5 months of age), control mice showed significant hypergammaglobulinemia,

with IgG-2a predominating (see Figure 6). In the initial studies with varied DMG concentration together with Perna, IgG-2a level in mice serum for the three different treatment groups were compared (see Figure 5). The lowest concentration of DMG (50mM) with Perna seems to have no effect on immunoglobulin level. The last two concentrations of DMG (100 & 200 mM) with Perna both show immunomodulation effect on IgG-2a level and decreased the amount of immunoglobulin level (see Figure 5). However, the higher concentration level of DMG (100 mM and 200 mM) made a significant change when compared to control groups (see Figure 7). DMG and Perna individually showed a significant immunomodulation effect on IgG-2a level by week 8. But an even more significant effect was observed when DMG and Perna were combined as immunomodulators, thus, providing a synergistic effect (see Figure 7).

Similar results were observed in subsequent studies with DMG (150 mM) held constant while the Perna amount was varied (see Figure 8). The treatment group with average amount of 41.7% Perna together with DMG showed a significant drop in IgG-2a level compared to control groups (see Figure 9).

Immunofluorecense microscopy

Frozen kidney sections from male 22-week-old mice treated with DMG and Perna for 15 weeks were stained with FITC-labelled antibodies against immunoglobulins. All control mice displayed heavy deposits of immunoglobulins in glomeruli. In contrast, approximately 50% of DMG and Perna treated mice showed little or no immunoglobulin deposits in glomeruli. In the remaining experimental animals immune deposition was plainly observed but at very low levels compared to the controls.

DISCUSSION

The MRL lpr/lpr mouse strain spontaneously develops an autoimmune disease resembling human SLE. It is the prevalent model for human SLE. The disease is characterized by immune complex-mediated glomerulonephritis, enlargement of spleen and lymph nodes, production of various auto antibodies such as anti-DNA antibodies and rheumatoid factors (RF) (see Vyse,T.J. and B.I. Kotzin, 1998, *Annu. Rev. Immunol.* 16:307).

MRL lpr/lpr mice were used in this study to examine the effect of the immunomodulation substances dimethylglycine (DMG) and Perna canaliculus on the progression of the SLE-like disease. Several immunomodulating drugs have been tested for their effects on the clinical course of SLE in MRL lpr/lpr mice. Cyclophosphamide (CYC) was established in 1984 as an effective inhibitor of lymphadenopathy, arthritis and nephritis in MRL lpr/lpr mice (see Smith, H. R. et al. 1984, *Clin. Immunol. Immunopathol.* 30, 51), and 2 years later it was shown that Linomide also exhibited disease-retarding properties comparable to that of CYC in these mice (Tarkowski,A. et.al., 1986, *Arthritis and Rheumatol.* 29:1405). Conflicting results were reported after treatment of MRL lpr/lpr mice with cyclosporine A (CsA). While one study showed no demonstrable effect of CsA (Burden, J. H. et al., 1986, *Scand. J. Immunol.* 24, 405) another revealed amelioration of immunopathology and prolonged survival (Mountz, J. D., 1987, *J. Immunol.* 138, 157).

Dimethylglycine (DMG), a tertiary amino acid, has had wide acceptance as a non-fuel nutrient; presumably it enhances oxygen utilization by tissue. A double-blind study in 20 human volunteers showed a fourfold increase in antibody response to pneumococcal vaccine in those receiving DMG orally as compared with controls. The *in vitro* responses of lymphocytes from patients with diabetes and those with sickle cell disease to

phytohemagglutinin were increased almost threefold after addition of DMG. Similar results suggest that DMG enhances both humoral and cell-mediated responses in humans (Graber, C. D. et al., 1981, *J. Infec. Dis.* 143, 101). Unlike synthetic immunomodulators that must be laboratory derived and thoroughly tested, DMG is present in low levels in animals and in foods (Mackenzie, C.G. and W. R. Frisell, 1958, *J. Bio. Chem.* 232 : 417). Enzyme systems in the body effectively convert the substance into metabolites that are either used by the body or are safely extracted. On the other hand, a Perna extract of the stabilized New Zealand green-lipped mussel powder has shown significant anti-inflammatory activity when given to animals and humans (Miller, T. E., and D. Ormand, 1980, *N. Z. Med. J.*, 92, 187).

By our invention, it has been demonstrated that DMG and Perna is an effective combination for treatment of nephritis in MRL lpr/lpr mice. The therapeutic efficacy of DMG is synergistically enhanced by using Perna at the same time. It has been also demonstrated that DMG or Perna individually will significantly affect the levels of I_gG_2 in lupus mice but, when they are administered together (see Figures 7 and 10), significantly amplified the down regulation of IgG-2a as compared to the DMG or Perna alone. The use of MRL lpr/lpr mice, genetically prone to develop and subcumb to a lupus-like disease, in our study clearly demonstrates the prophylactic and therapeutic effect of the combination of DMG and Perna on lupus nephritis.

In this study, we found that oral administration of the combination of DMG and Perna significantly suppressed the development of glomerulonephritis and lymphadenopathy (Tables 8-11) in MRL lpr mice. These results suggested that the combination of DMG and Perna ameliorates the lupus-like autoimmune disorders by modulating Thl, which in turn results in skewing positively of the immune response in MRL/lpr mice. It was reported that IFN-γ promoted the secretion of IgG-2a and IgG3, and IL-4 induced IgGl production

(Takahashi, S. et al., 1991, *J. Immunol.*, 147, 515). The DMG and Perna combination clearly suppressed the production of IgG-2a level in serum (see Figure 6), but did not change that of IgGl (See Figure 11). Interestingly, DMG and Perna increased the production of IgG3 in serum (see Figures 11 and 12). Therefore, the total concentration of immunoglobulin essentially remained the same.

DMG and Perna showed significant effects on lymphadenopathy. Average lymph nodes weight for mice treated with the highest concentration of DMG (200 mM) plus Perna was reduced to half compared to control mice. This reduction was also seen in other treatment groups with 100 mM DMG but not as significant as in 200 mM treated mice. Mice treated with 50 mM DMG plus Perna showed no significant change in their lymph node weight. Animals treated with different concentrations of Perna plus 150 mM DMG showed similar results but the reduction is not as highly significant as that caused by 200 mM treated mice (see Tables 10 and 11). DMG and Perna showed no immunomodulation effect on spleen weight, kidney weight and BUN score.

In conclusion, we have shown that the immunomodulation resulting from the combination of DMG and Perna significantly reduces the progression of glomerulonephritis and pathological IgG-2a antibody levels. Being natural compounds, it is expected that fewer serious side effects might occur with DMG and Perna. The study also revealed that the combination significantly reduced the pathological enlargement of the lymph nodes. Such properties shown in the prevalent mouse model is of high significance to treating SLE patients with glomerulonephritis. Such significant property of the DMG and Perna combination could not have been expected by one viewing the previous reports on uses of DMG and Perna.

Certain facts indicate to the contrary of an advantage of the combination. For example, it was known that DMG preferentially stimulates Th1 responses, including increasing the levels of interferon gamma and, thereby, secondarily IgG-2a, rather than decreasing IgG-2a. Tumor necrosis factor alpha and Il-1 have also been reported to be elevated by DMG. In our laboratory, we previously observed that the administration of DMG to collagen-11 induced arthritic mice exacerbated the level of inflammation. Also, as noted above, DMG alone at concentrations of 200 mM, which is readily tolerated by normal mice, caused severe cachexia and symptoms of shock in some lupus prone MRL lpr/lpr mice. Therefore, the administration of DMG, either alone or in combination with other substances, such as Perna powder, to animals or humans prone to the development or active progression of lupus-like diseases could have been considered in the art as disadvantageous rather than advantageous.

Mode of Administration

I have read the Declaration of my co-inventor, Dr. Roger V. Kendall, signed June 18, 2002, and submitted earlier in the prosecution of this application. I concur with the statements made therein and also independently conclude that one of ordinary skill in the art, at the time of our invention, upon reading our application, would find a description in the application of compositions of DMG and Perna both in a form suitable for enteral but not parenteral administration.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed true; and further that these statements were made with the knowledge that willful false statements and the like so made

are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/19/03
Date

John Lawson
Dr. John Lawson

Table 1

Treatment Groups I

Group No.	Mouse strain	n	Treatment	Age of therapy
1 (TA)	MRL MPJ lpr	9	50 mM DMG & perna-mouse chow mixture (41.7% Perna)	7-22 weeks
2 (TB)	MRL MPJ lpr	9	100 mM DMG & perna-mouse chow mixture (41.7% Perna)	7-22 weeks
3 (TC)	MRL MPJ lpr	9	200mM DMG & perna-mouse chow mixture (41.7% Perna)	7-22 weeks

Table 2

Treatment Groups II

Group No.	Mouse strain	n	Treatment	Age of therapy
1 (T1)	MRL MPJ lpr	9	150 mM DMG & perna-mouse chow mixture #1 (20.85% Perna)	8-22 weeks
2 (T2)	MRL MPJ lpr	9	150 mM DMG & perna-mouse chow mixture #2 (41.7% Perna)	8-22 weeks
3 (T3)	MRL MPJ lpr	9	150mM DMG & perna-mouse chow mixture #3 (83.4% Perna)	8-22 weeks

Table 3

Control groups I

Group No.		Mouse strain	n	Treatment	Age of therapy
1 (CH)		MRL MPJ lpr	3	Distilled water/ standard mouse chow	8-22 weeks
2 (CP)		MRL MPJ lpr	3	Distilled water/ Perna-mouse chow mixture	8-22 weeks
3 (CD)	CD1	MRL MPJ lpr	3	50 mM DMG/ standard mouse chow	7-22 weeks
	CD2	MRL MPJ lpr	2	100 mM DMG/ standard mouse chow	7-22 weeks
	CD3	MRL MPJ lpr	2	200 mM DMG/ standard mouse chow	7-22 weeks

Table 4

Control groups II

Group No.		Mouse strain	n	Treatment	Age of therapy
CH1 (water control)		MRL MPJ lpr	3	Distilled water/ standard mouse chow	8-22 weeks
CH2		MRL MPJ lpr	2	Distilled water/ standard mouse chow	8-22 weeks
CD (DMG control)		MRL MPJ lpr	2	DMG 150 mM Standard mouse chow	8-22 weeks
CP (Perna control)	CP1	MRL MPJ lpr	2	Distilled water perna-mouse chow mixture #1 (20.85% Perna)	8-22 weeks
	CP2	MRL MPJ lpr	2	Distilled water perna-mouse chow mixture #1 (41.7% Perna)	8-22 weeks
	CP3	MRL MPJ lpr	2	Distilled water perna-mouse chow mixture #3 (83.4% Perna)	8-22 weeks

Table 5. Average consumption of DMG.

Average water intake (ml) per mice per day						
Cage #	**Week 0**	**Week 1**	**Week 4**	**Week 8**	**Week 12**	**Week 16**
TA	9.7	12.5	12.5	13.1	14.1	20.8
TB	9.8	12.6	12.1	13.8	12.4	20
TC	9.8	13.0	13.8	14.3	12.1	25
CH	10.9	10.7	8.8	10	10	10
CP	8.7	10.8	8.8	11.6	22.5	20
CD1	9.1	7.8	8.8	7.7	10	-
CD2	8.7	11.3	11.6	10.8	15	15
CD3	10.8	12.5	13.7	12.0	12.5	12.5

A= 50 mM DMG
B= 100 mM DMG
C= 200 mM DMG

Table 6

Average consumption of Perna (gram)			
Cage #	Week 1	Week 2	Week4
T1	7.5	7.4	7.3
T2	8.2	7.1	7.3
T3	7.3	7.2	7.9
CH			
CD			
CP1			
CP2			
CP3			

Table 7

Average consumption of DMG (ml)			
Cage #	Week 1	Week 2	Week4
T1	12.9	12.7	12.2
T2	19.6	17.5	18.6
T3	23.2	25.3	23.6
CH			
CD			
CP1			
CP2			
CP3			

Table 8. Effect of different concentrations of DMG with 41.7% Perna on Lymphoid organ weights in MRL lpr mice at age 16 weeks.

	n	Avg weight of lymph nodes	Avg weight of spleen
TA (50 mM DMG)	4	1.0 ±0.4	0.27 ± 0.05
TB (100mM DMG)	4	0.9 ± 0.3	0.35 ± 0.05
TC (200 mM DMG)	4	0.76 ±0.2	0.25 ± 0.1
Control	6	1.5 ± 1.2	0.3 ± 0.1

Lymph nodes (axillary, inguinal, cervical and mesenteric) and spleen weights are indicated in grams

Table 9. Effect of different concentrations of DMG with 41.7% Perna on Lymphoid organ weights in MRL lpr mice at age 22 weeks.

	n	Avg weight of lymph nodes	Avg weight of spleen
TA (50 mM DMG)	4	1.8 ± 0.8	0.5 ± 0.1
TB (100mM DMG)	5	1.5 ± 0.5	0.5 ± 0.1
TC (200 mM DMG)	3	1.2 ± 0.4	0.5 ± 0.2
Control	5	2.0 ± 0.4	0.4 ± 0.1

Lymph nodes (axillary, inguinal, cervical and mesenteric) and spleen weights are indicated in grams

Table 10. Effects of different concentration of Perna on Lymphoid organ weights in MRL lpr mice at age 16 weeks

	n	Avg weight of lymph nodes	Avg weight of spleen
T1	4	1.25 ± 0.2	0.25 ± 0.05
T2	4	1.25 ± 0.5	0.32 ± 0.09
T3	4	1.35 ± 0.3	0.27 ± 0.05
Control	6	1.65 ± 0.4	0.28 ± 0.04

Lymph nodes (axillary, inguinal, cervical and mesenteric) and spleen weights are indicated in grams

Table 11. Effects of different concentration of Perna on Lymphoid organ weights in MRL lpr mice at age 22 weeks

	n	Avg weight of lymph nodes	Avg weight of spleen
T1	5	2.0 ± 0.7	0.58 ± 0.2
T2	4	1.82 ± 1.0	0.42 ± 0.2
T3	4	1.57 ± 0.6	0.37 ± 0.09
Control	5	1.9 ± 0.5	0.52 ± 0.1

Lymph nodes (axillary, inguinal, cervical and mesenteric) and spleen weights are indicated in grams

Table 12. Effects of different concentration of DMG on Kidney pathology in MRL MPJ lpr mice at the age of 16 weeks

	n	Avg. BUN	Avg. Kidney WT
TA (50 mM DMG)	4	2.5	0.75 ± 0.05
TB (100mM DMG)	4	3.75	0.95 ± 0.2
TC (200 mM DMG)	4	2.25	0.77 ± 0.1
Control	6	2.6	0.65 ± 0.05

Table 13. Effects of different concentration of DMG on Kidney pathology in MRL MPJ lpr mice at the age of 22 weeks

	n	Avg. BUN	Avg. Kidney WT
TA (50 mM DMG)	4	4	0.9 ± 0.2
TB (100mM DMG)	5	3.2	1.1 ± 0.1
TC (200 mM DMG)	3	3.3	0.9 ± 0.05
Control	5	4	0.9 ± 0.1

Table 14. Effects of different concentration of Perna on Kidney pathology in MRL MPJ lpr mice at the age of 16 weeks

	n	Avg. BUN	Avg. Kidney WT
T1	4	3.25	0.72 ± 0.05
T2	4	3	0.8 ± 0.08
T3	4	3.2	0.95 ± 0.1
Control	6	2.3	0.78 ± 0.09

Table 15. Effects of different concentration of Perna on Kidney pathology in MRL MPJ lpr mice at the age of 22 weeks

	n	Avg. BUN	Avg. Kidney WT
T1	5	4	1.0 ± 0.1
T2	4	3.5	0.97 ± 0.09
T3	4	4	1.5 ± 0.6
Control	5	4	1.0 ± 0.1

Table 16

	n *	Initial body weight (g)	Increased weight at week 8 of Exp.	Increased weight at week 16 of Exp.
TA	9 (5)			
TB	9 (5)			
TC	9 (5)			
Control	13 (6)			

* Half of the animals were sacrificed at week 8 and the rest at week 16 of experiment. The number in paranthesis is showing "n" for week 16 of experiment

Table 17. Effect of different concentration of Perna on body weight of MRL mice.

	n *	Initial body weight (g)	Increased weight at week 8 of Exp.	Increased weight at week 16 of Exp.	
T1	9 (5)		6.3 ± 2.4	10.4 ± 2.6	
T2	9 (5)		6.7 ± 0.7	5.8 ± 5.2	
T3	9 (5)		4.2 ± 1.2	2.3 ± 5.1	
Control	13 (6)		5.6 ± 2.8	6.8 ± 4.6	

* Half of the animals were sacrificed at week 8 and the rest at week 16 of experiment. The number in paranthesis is showing "n" for week 16 of experiment

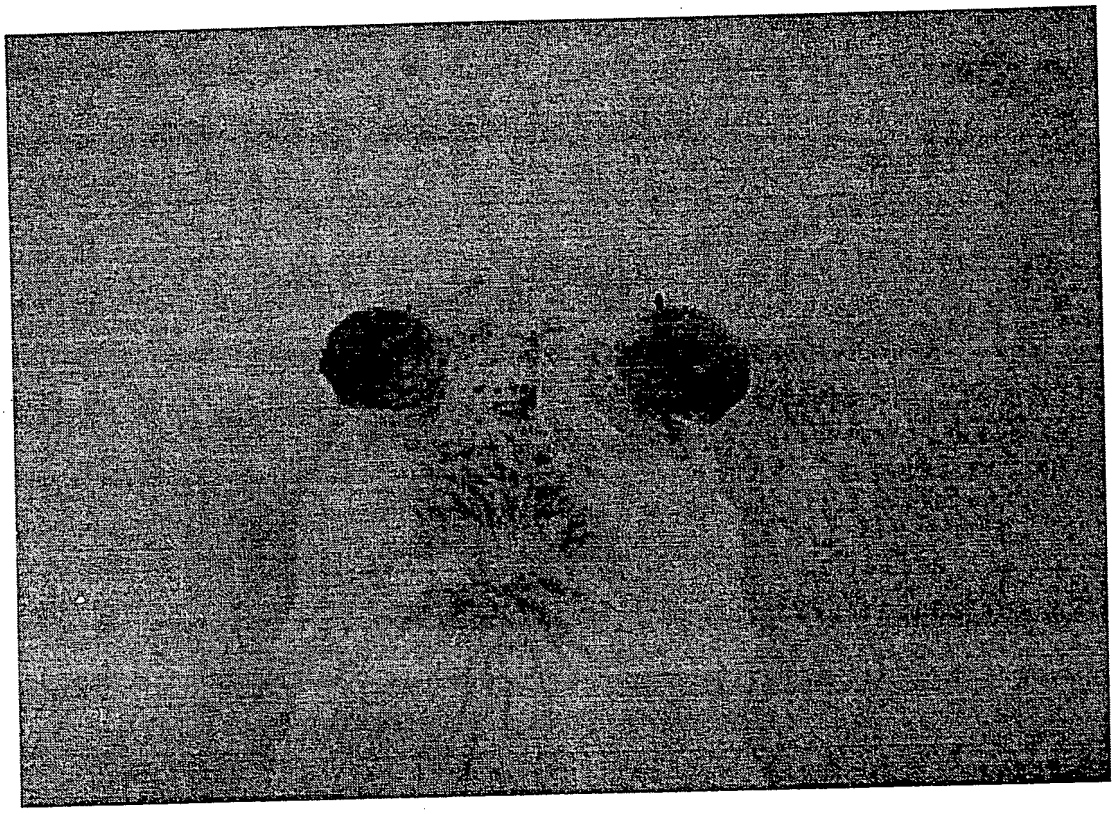

Figure 1.

Figure 2.

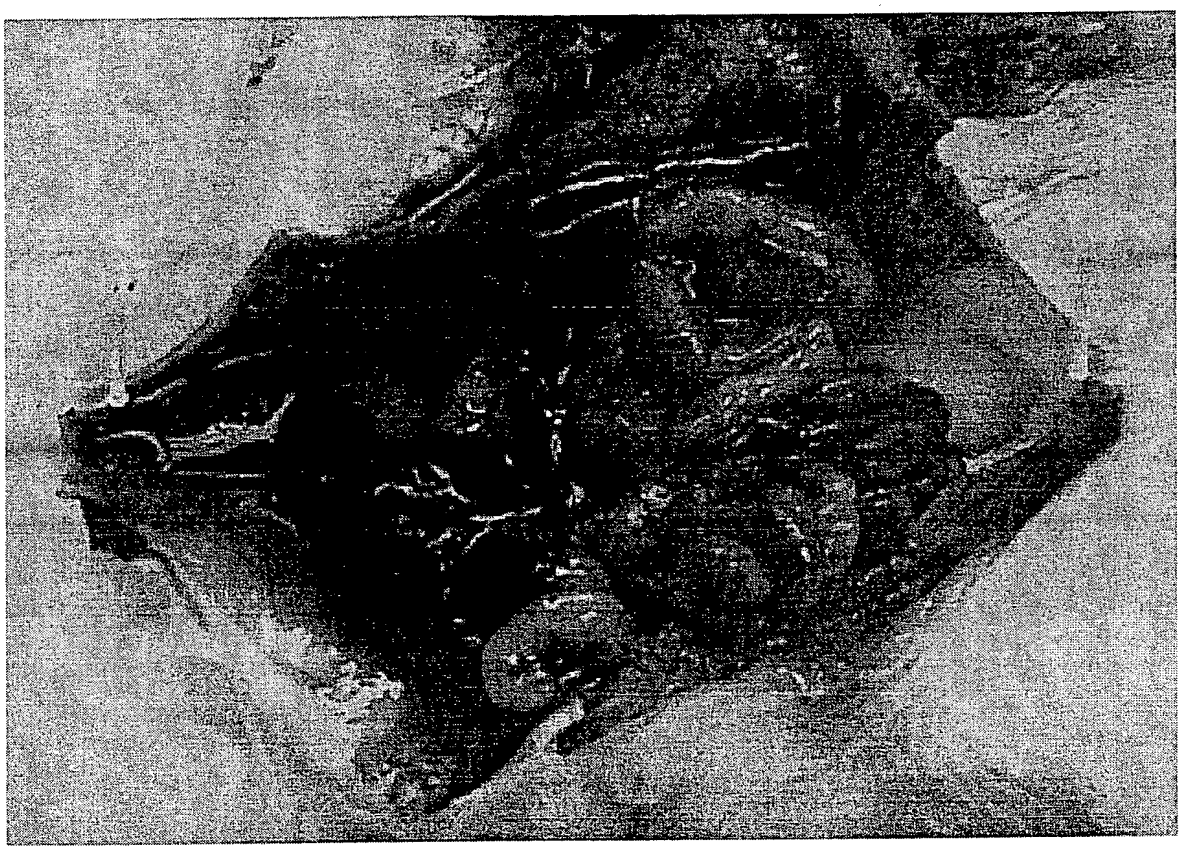

Figure 3.

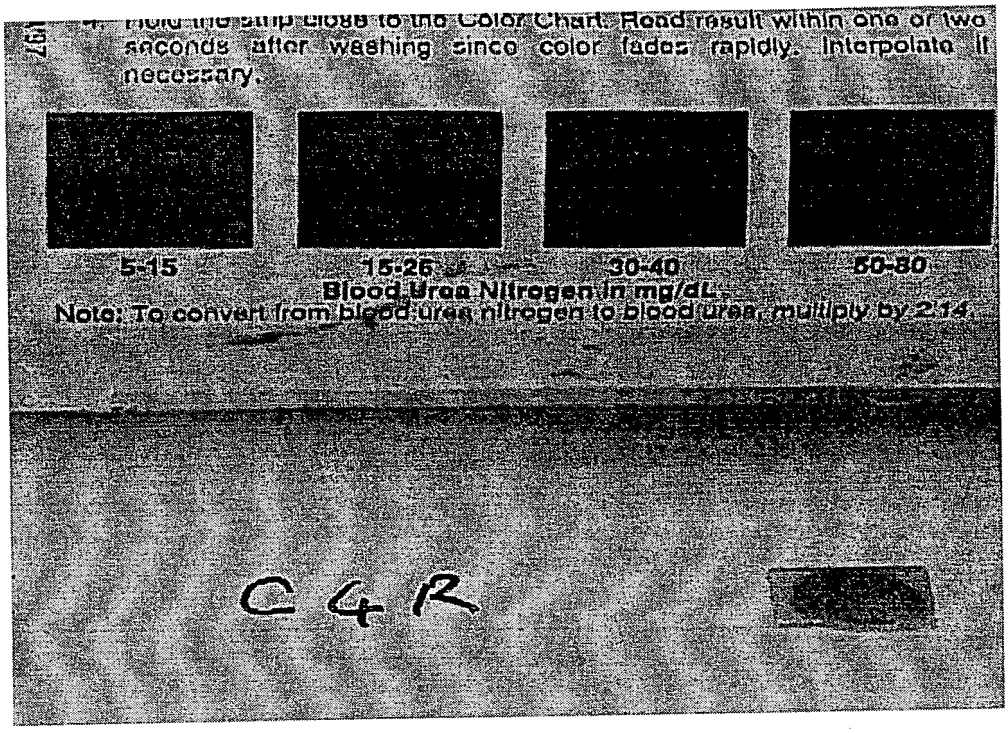
4. Hold the strip blobs to the Color Chart. Read result within one or two seconds after washing since color fades rapidly. Interpolate if necessary.
5-15
15-25
30-40
50-80
Blood Urea Nitrogen in mg/dL
Note: To convert from blood urea nitrogen to blood urea, multiply by 2.14
C 4 R

Figure 4.

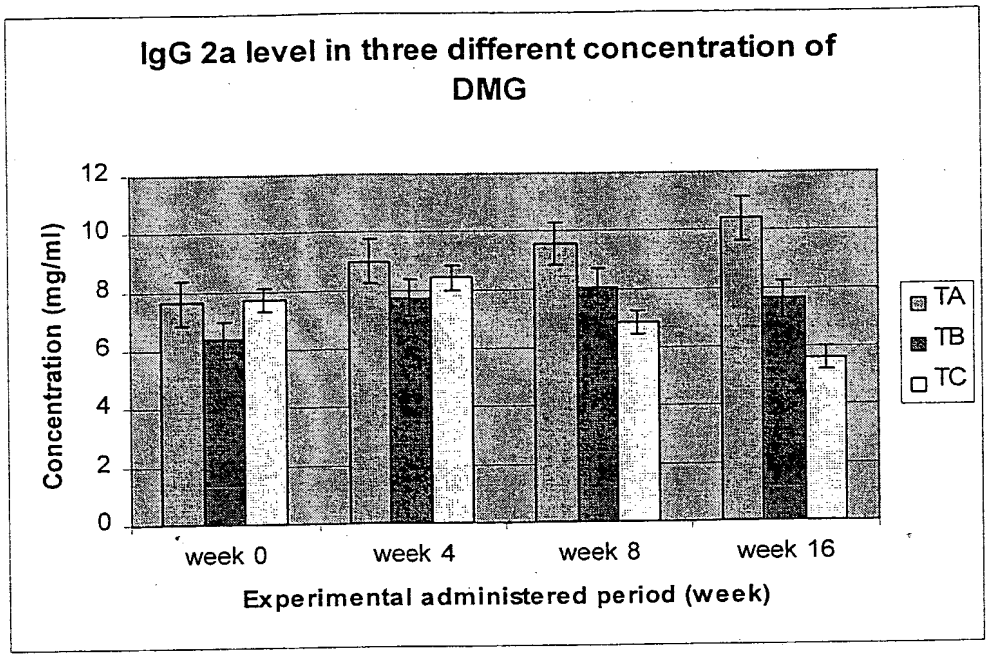

Figure 5. Effects of 3 different concentration of DMG on serum levels of immunoglobulin antibody IgG 2a in MRL lpr/lpr mice treated from 8 weeks of age. Groups of 9 MRL *lpr/lpr* mice were treated with DMG + Perna for 16 weeks. Sera was collected every 4 weeks and assayede by ELISA for IgG isotypes. All the treatment groups got constant concentration of Perna (41.7% Perna mixed with standard mouse chow). TA= Treated mice with 50 mM DMG + Perna, TB= Treated mice with 100 mM DMG + Perna, and TC= Treated mice with 200 mM DMG + Perna ($P < 0.05$).

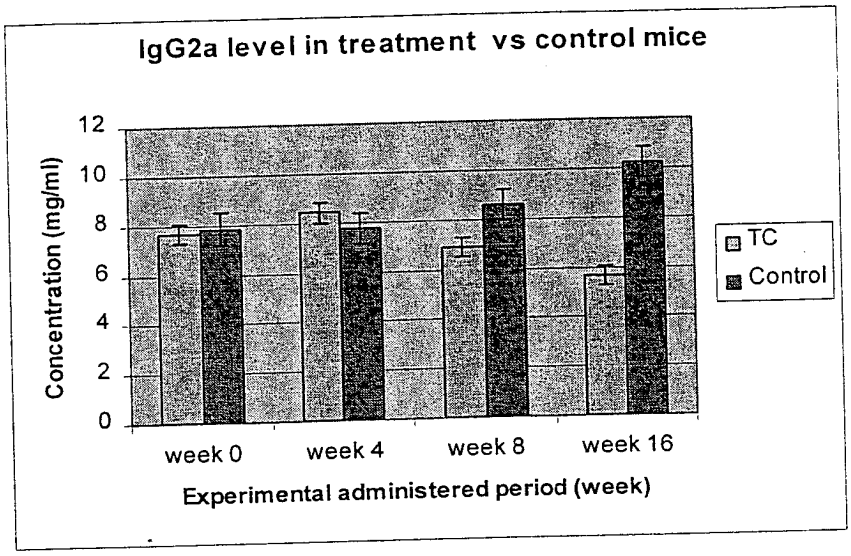

Figure 6. IgG2a level in treated mice with 200 mM DMG + Perna is compared to control mice (not treated animals). Starting at 8 weeks of age (week 0 of experiment), MRL *lpr/lpr* mice were treated with DMG + Perna for 16 weeks. Sera was collected every 2 weeks and assayede by ELISA for IgG isotypes. MRL *lpr/lpr* mice exhibit the lupus at 12 weeks of age (week 4 of experiment). The treatment (DMG + Perna) shows the reduction of serum level of IgG2a in MRL mice at week 8 of experiment and the reduction is more significant at late stage of experiment. $0.05<P<0.1$

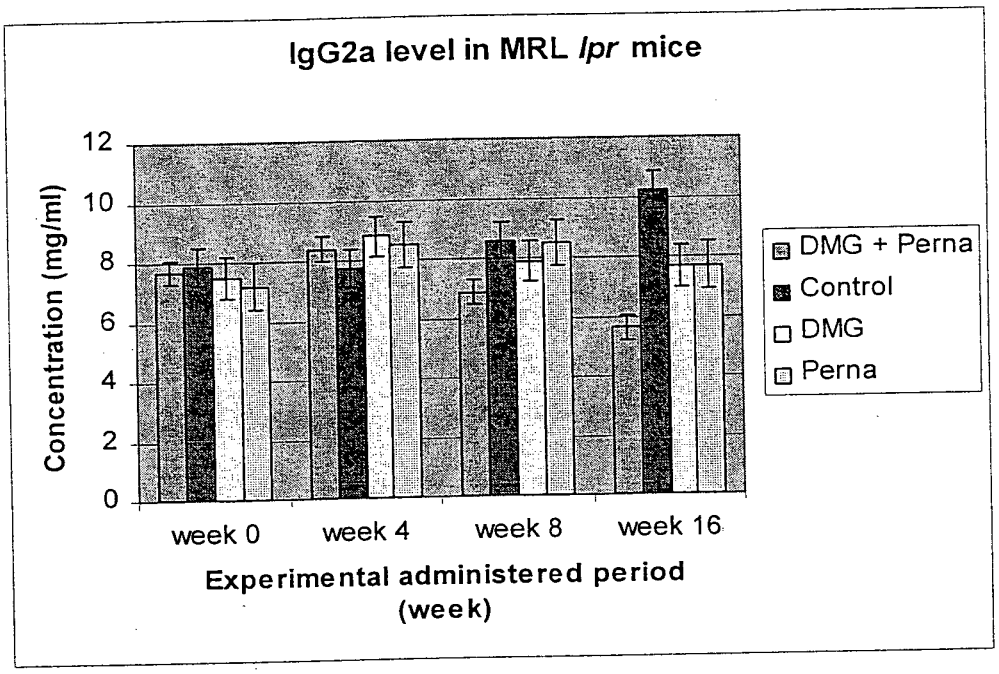

Figure 7. IgG2a level in treated mice with 200 mM DMG + Perna is compared to control (not treated animals), DMG treated Perna treated mice. Treatment was started at 8 weeks of age and continued for 16 weeks. Sera were collected every 4 weeks and assayed by ELISA for IgG isotypes. $P<0.1$

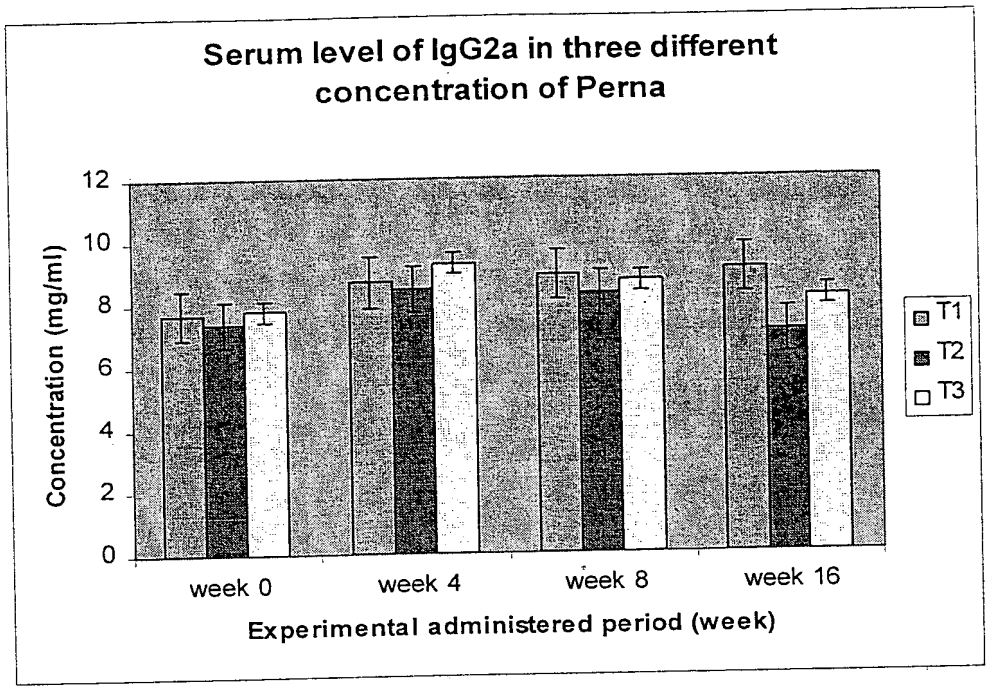

Figure 8. Effects of 3 different concentration of Perna on serum levels of immunoglobulin antibody IgG2a in MRL lpr/lpr mice treated from 8 weeks of age. Groups of 9 MRL *lpr/lpr* mice were treated with DMG + Perna for 16 weeks. Sera was collected every 4 weeks and assayede by ELISA for IgG isotypes. All the treatment groups got constant concentration of DMG (150 mM). T1= 20% Perna, T2= 41% Perna, and T3= 83% Perna mixed with standard mouse chow.

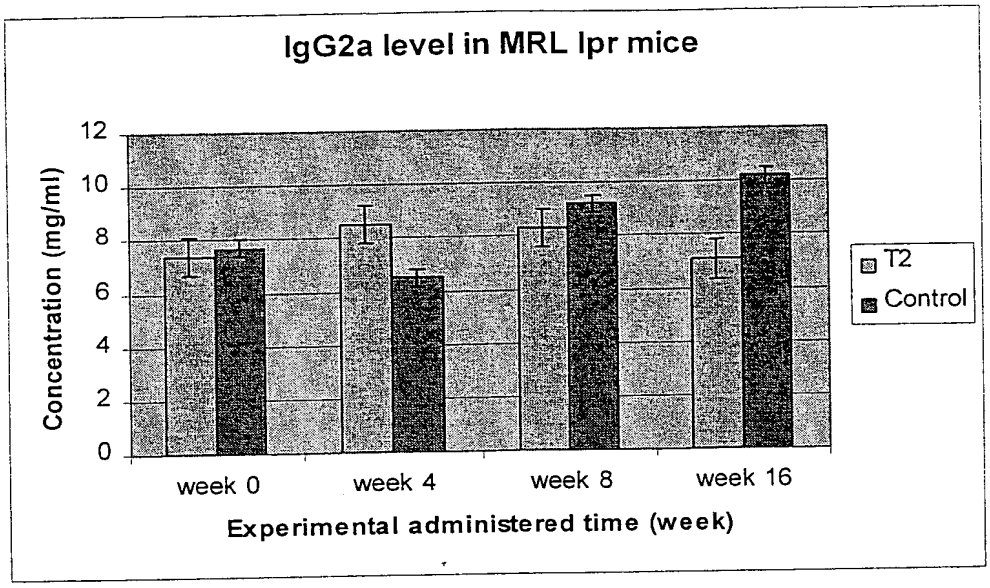

Figure 9. IgG2a level in treated mice with 150 mM DMG + 41.7% Perna is compared to control mice (not treated animals). Starting at 8 weeks of age (week 0 of experiment), MRL *lpr/lpr* mice were treated with DMG + Perna for 16 weeks. Sera was collected every 4 weeks and assayede by ELISA for IgG isotypes. MRL *lpr/lpr* mice exhibit the lupus at 12 weeks of age (week 4 of experiment). The treatment (DMG + Perna) shows the reduction of serum level of IgG2a in MRL mice at week 4 of experiment and continued until late stage of experiment. $0.05<P<0.1$

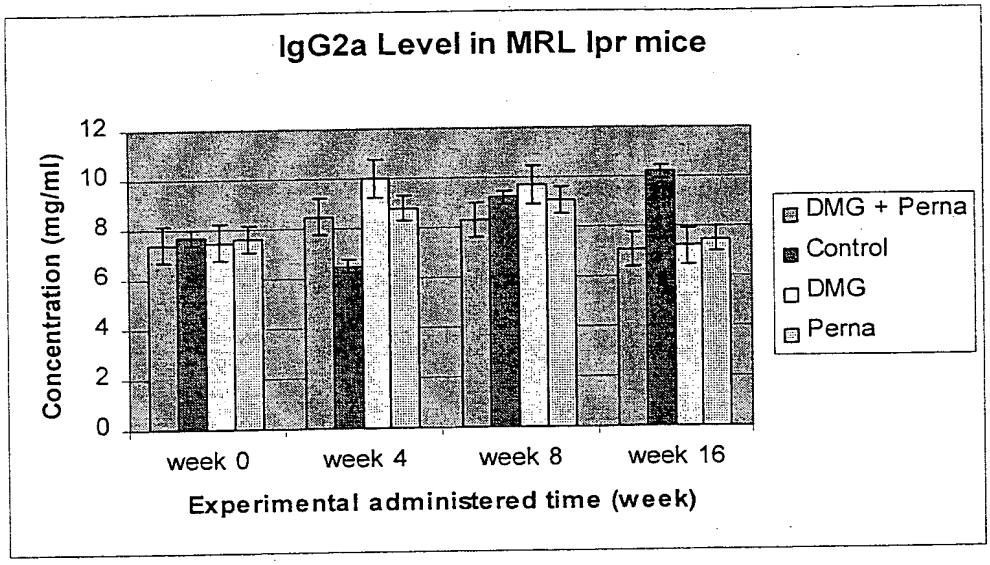

Figure 10. Serum levels of IgG2a in treated MRL *lpr/lpr* mice vs controls. IgG2a level in treated mice with 150 mM DMG + 41.7% Perna is compared to control (not treated animals), DMG treated and Perna treated mice. Treatment was started at 8 weeks of age and continued for 16 weeks. Sera were collected every 4 weeks and assayed by ELISA for IgG isotypes. $P>0.1$

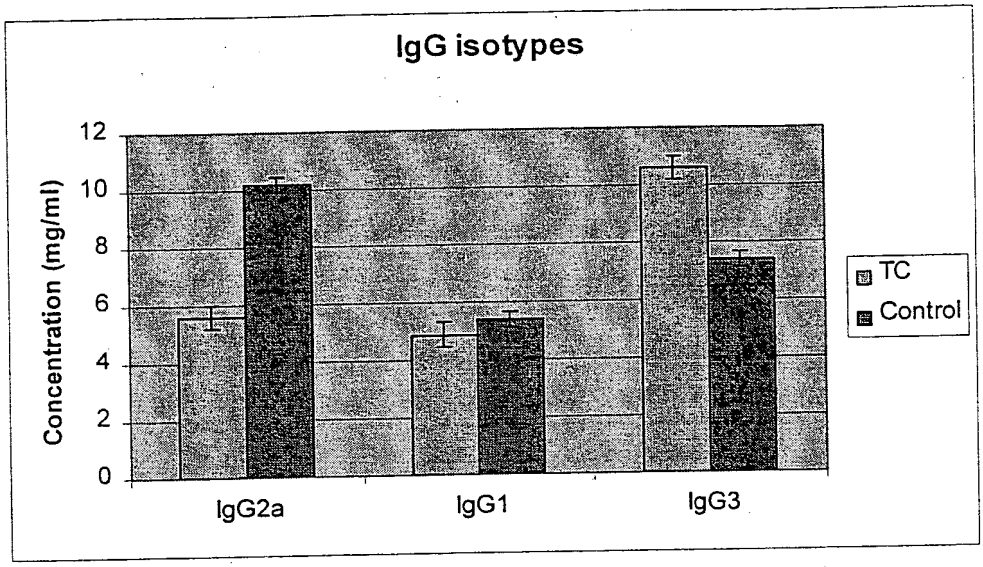

Figure 11. Effect of 200 mM DMG + Perna (41.7% Perna mixed with standard mouse chow) on IgG isotypes in MRL *lpr/lpr* mice.

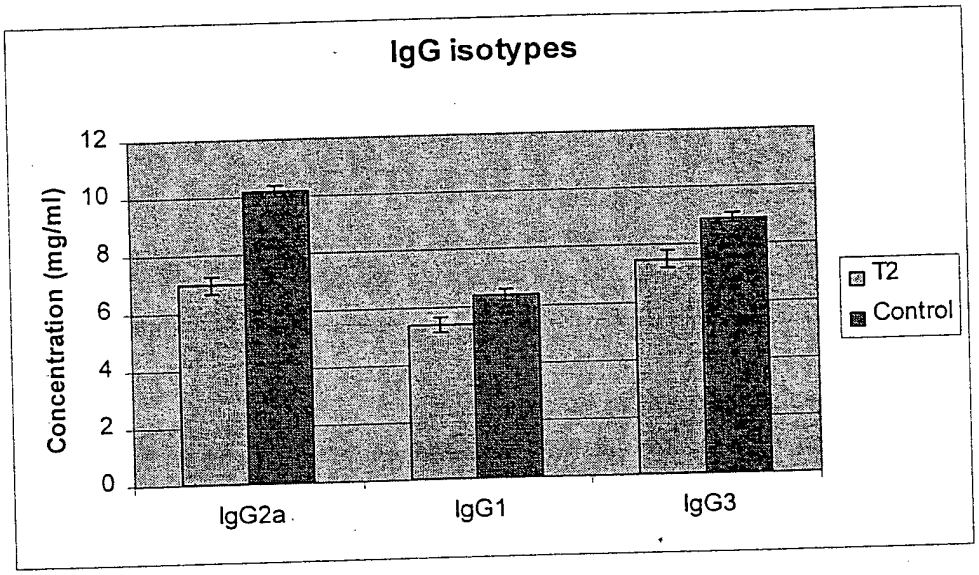

Figure 12. Effect of 150 mM DMG + Perna (41.7% Perna mixed with standard mouse chow) on IgG isotypes in MRL *lpr/lpr* mice.

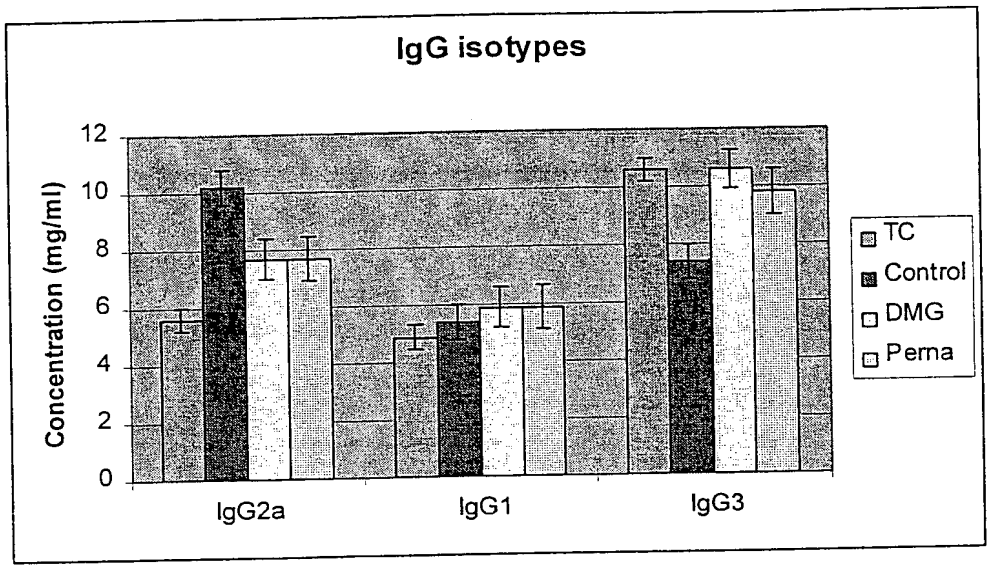

Figure 13. IgG isotypes in treated MRL *lpr/lpr* mice vs controls at 22 weeks.

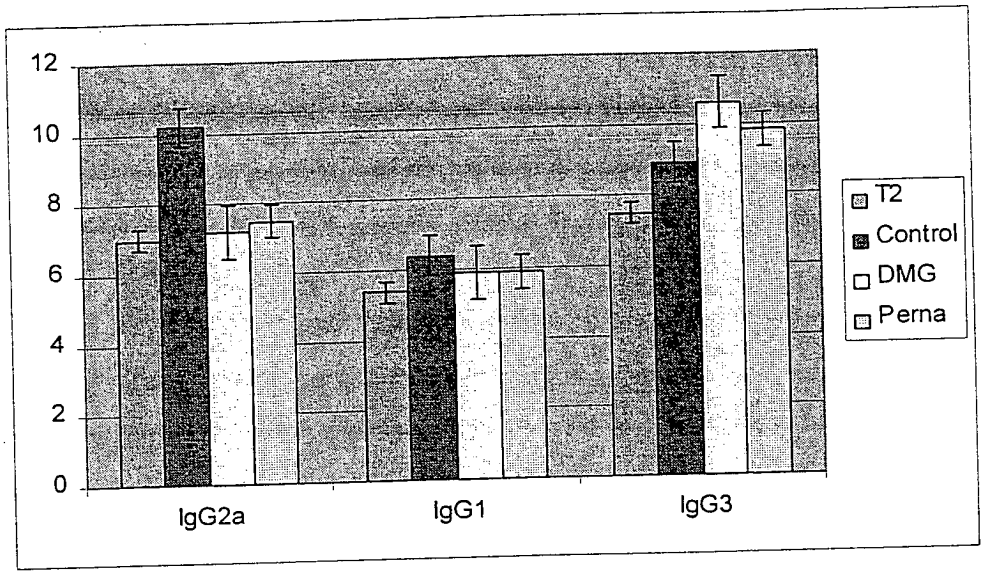

Figure 14. . IgG isotypes in treated MRL *lpr/lpr* mice vs controls.

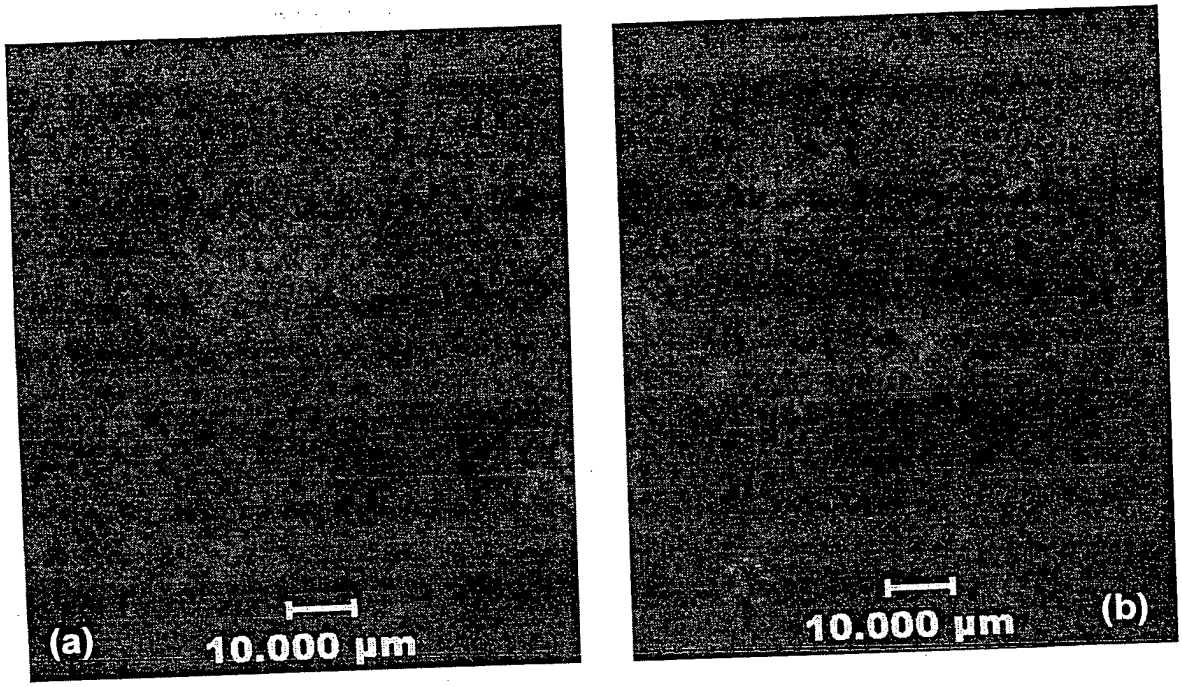

Figure 15. Comparison of glomerular pathologic findings and immunoflurescent deposits from a treated animal with 200 mM DMG and 41% Perna miture (a), and a control mouse (b).

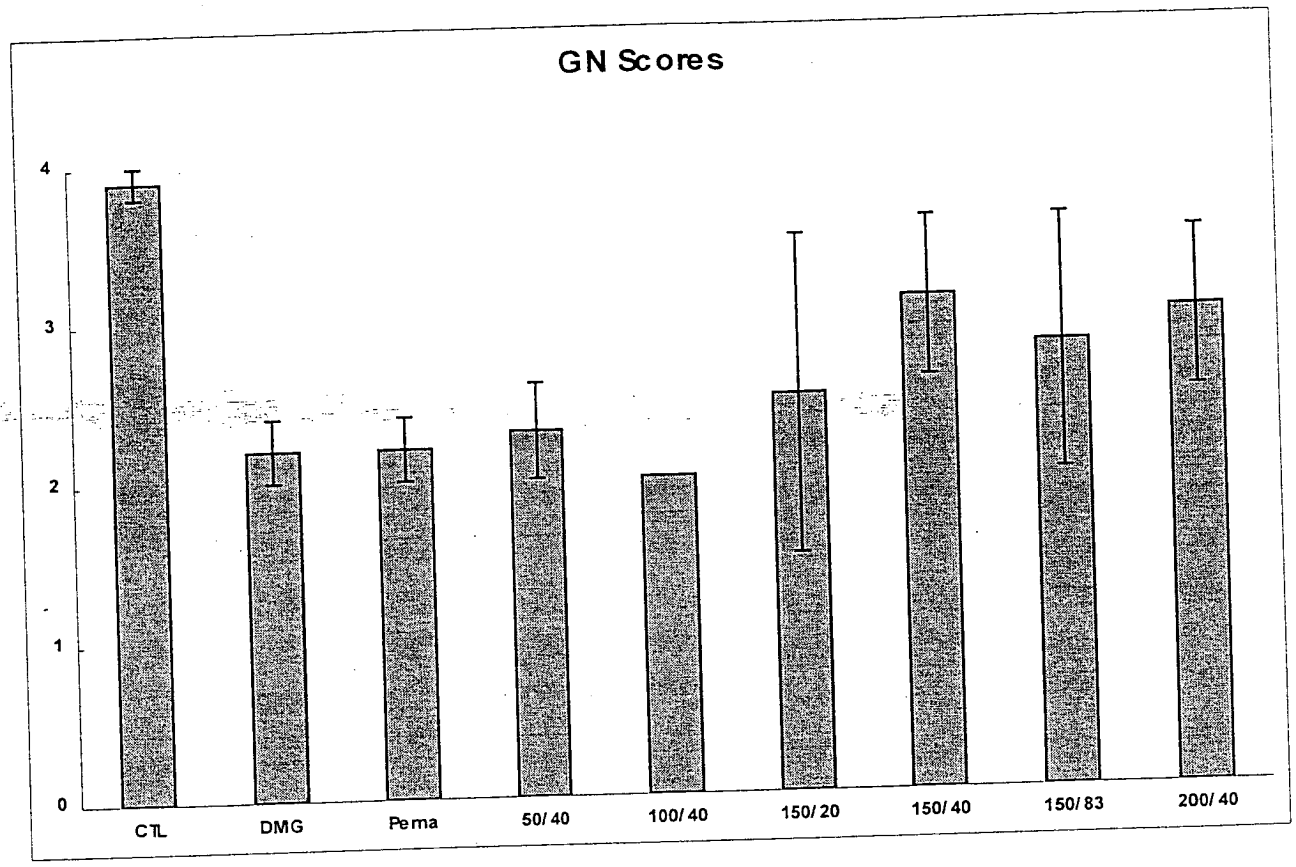

Figure 16. Glomerunephritis was shown by using periodic acid Schiff reagent (PAS) technique. Glomerular inflammatory cells were graded "rare" when 0-1 cells were seen per glomerular, and "occasional" when 1-2 cells were seen per glomerulus.

BIOSKETCH

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Academic Background

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1978 - 1980	Associate Professor Microbiology, Clemson University, Clemson, SC
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Pertinent Publications and Patents

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